

Nucleotide Sequence and Characterization of the *Staphylococcus aureus norA* Gene, Which Confers Resistance to Quinolones

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The *norA* gene cloned from chromosomal DNA of quinolone-resistant *Staphylococcus aureus* TK2566 conferred relatively high resistance to hydrophilic quinolones such as norfloxacin, enoxacin, ofloxacin, and ciprofloxacin, but only low or no resistance at all to hydrophobic ones such as nalidixic acid, oxolinic acid, and sparfloxacin in *S. aureus* and *Escherichia coli*. The 2.7-kb DNA fragment containing the *norA* gene had a long open reading frame coding for 388 amino acid residues with a molecular weight of 42,265, which was consistent with the experimental value of about 49,000 obtained on DNA-directed translation. The deduced NorA polypeptide has 12 hydrophobic membrane-spanning regions and is partly homologous to tetracycline resistance protein and sugar transport proteins. The uptake of a hydrophilic quinolone, enoxacin, by *S. aureus* harboring a plasmid carrying the *norA* gene was about 50% that by the parent strain lacking the plasmid, but it increased to almost the same level as that by the latter strain with carbonyl cyanide *m*-chlorophenyl hydrazone. On the other hand, the uptake of a hydrophobic quinolone, sparfloxacin, was similar in the two strains. These results suggest that the NorA polypeptide may constitute a membrane-associated active efflux pump of hydrophilic quinolones.

The increase in methicillin-resistant *Staphylococcus aureus* is a serious problem because only a few effective agents are clinically available. Some quinolones have been used for the treatment of methicillin-resistant *S. aureus* infections, but the emergence of quinolone resistance has been reported elsewhere (32). Unlike the mechanism underlying the quinolone resistance of gram-negative bacteria such as *Escherichia coli* (2, 7, 9, 11, 12, 15, 27, 31, 36-39) and *Pseudomonas aeruginosa* (4, 13, 16, 29, 30, 36, 40), the mechanism underlying staphylococcal resistance to quinolones remains obscure. Recently, a DNA fragment which confers fluoroquinolone resistance, the *norA* gene, was cloned from a quinolone-resistant clinical methicillin-resistant *S. aureus* isolate (33). Southern hybridization analysis showed that a DNA fragment derived from quinolone-susceptible *S. aureus* was hybridizable with the *norA* gene. Quinolone resistance due to the *norA* gene was dominant over quinolone susceptibility, being different from quinolone resistance due to the *gyrA* or *gyrB* gene of *E. coli*. To characterize the *norA* gene in detail, we sequenced the gene and analyzed the deduced NorA polypeptide. The influence of the *norA* gene upon quinolone uptake by *S. aureus* cells was also examined.

MATERIALS AND METHODS

Bacteria. *S. aureus* SA113 (*r*⁻ *m*⁻) and *E. coli* HB101 [F⁻ *hsd*20 (*r*⁻ *m*⁻) *recA13*], which are quinolone-susceptible strains, were used as host strains into which plasmids carrying the *norA* gene were introduced.

Plasmids. pTUS20 is a staphylococcal plasmid containing

a 5.5-kb *Hind*III fragment with the *norA* gene (33). pTUS1 is a derivative of *E. coli* plasmid pBR322 containing the above 5.5-kb *Hind*III fragment (33). Deletion plasmids were constructed from pTUS1 (Fig. 1). pTUS180 and pTUS829 contain a 3.2-kb *Kpn*I-*Hae*III fragment and a 2.7-kb *Kpn*I-*Sph*I fragment of pTUS1, respectively, which were blunt ended with T4 DNA polymerase and inserted at the *Eco*RV site of pBR322. pTUS206 contains a 1.8-kb *Mae*II fragment of pTUS1 at the *Cla*I site of pBR322, and pTUS207 contains a 1.2-kb *Dra*I fragment of pTUS1 at the *Eco*RV site of pBR322. Plasmid pKK232-8, a pBR322 derivative containing a promoterless chloramphenicol acetyltransferase gene, was purchased from Pharmacia LKB Biotechnology.

Materials. Nalidixic acid (20), enoxacin (23), norfloxacin (18), ofloxacin (10), ciprofloxacin (8), sparfloxacin (26), ¹⁴C-enoxacin (26.9 μ Ci/mg) and ¹⁴C-sparfloxacin (20 μ Ci/mg) were synthesized in our laboratories. Materials were purchased from the following sources: restriction endonuclease *Mae*II from Boehringer Mannheim GmbH; other enzymes and a sequencing kit from Takara Shuzo Co., Ltd.; [α -³²P] dCTP, L-[4,5-³H]leucine and a prokaryotic DNA-directed translation kit from Amersham International; sodium ampicillin from Meiji Seika Kaisha, Ltd.; lysozyme (grade I), RNase (type 1-A), carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), and carbenicillin from Sigma Chemical Co.; sodium methicillin from Banyu Pharmaceutical Co., Ltd.; and the other reagents (guaranteed grade) from Nacalai Tesque, Inc.

Preparation of plasmid DNA. Small-scale plasmid DNA isolation was carried out by the rapid boiling method described by Holmes and Quigley (14), and large-scale plasmid isolation was performed by the method of Wilkie et al. (35).

DNA sequencing. DNA sequences were determined by the dideoxy chain termination method by using bacteriophage

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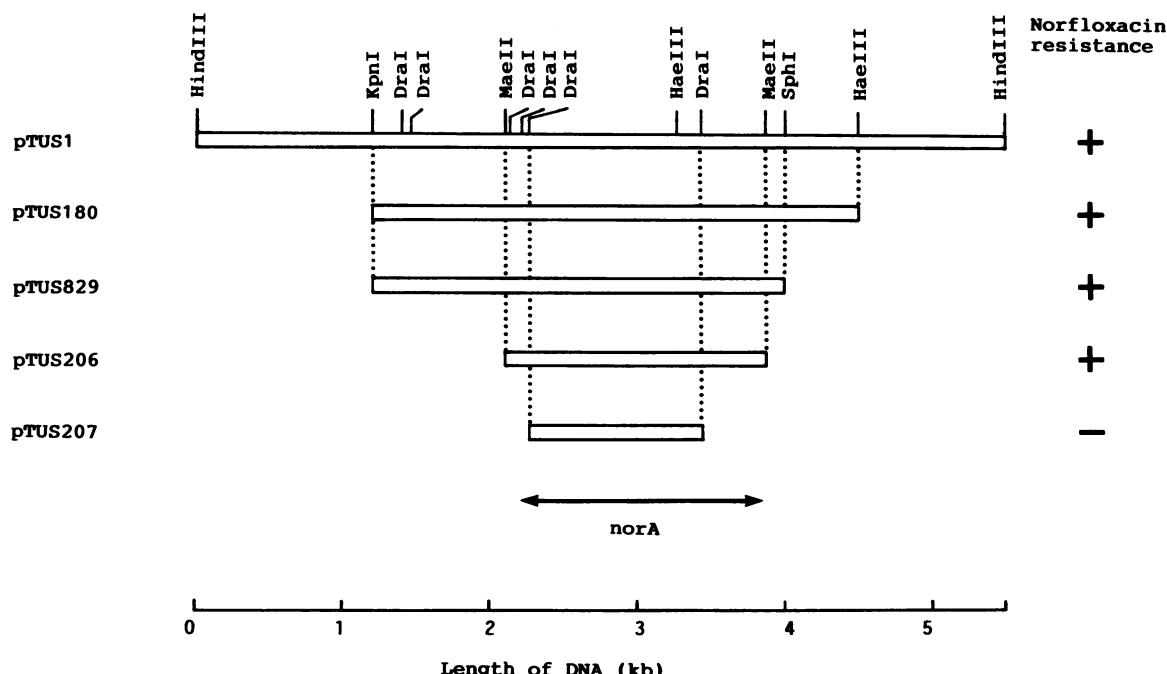


FIG. 1. Deletion plasmids of pTUS1 containing a 5.5-kb *HindIII* *S. aureus* chromosomal fragment. Only DNA inserts are shown. +, Plasmid possesses the ability to confer norfloxacin resistance.

M13mp18 and M13mp19 vectors (25). Other DNA techniques were used essentially as described by Maniatis et al. (22).

Expression of the norA gene. The 7.1-kb *BamHI*-cleaved pTUS829 DNA fragment containing the norA gene (ca. 0.2 $\mu\text{g}/\text{ml}$) was expressed through DNA-directed translation

with the Amersham International kit according to the manufacturer's manual, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to check its molecular weight.

Uptake of quinolones by *S. aureus* cells. The uptake of quinolones by *S. aureus* SA113 and *S. aureus* SA113

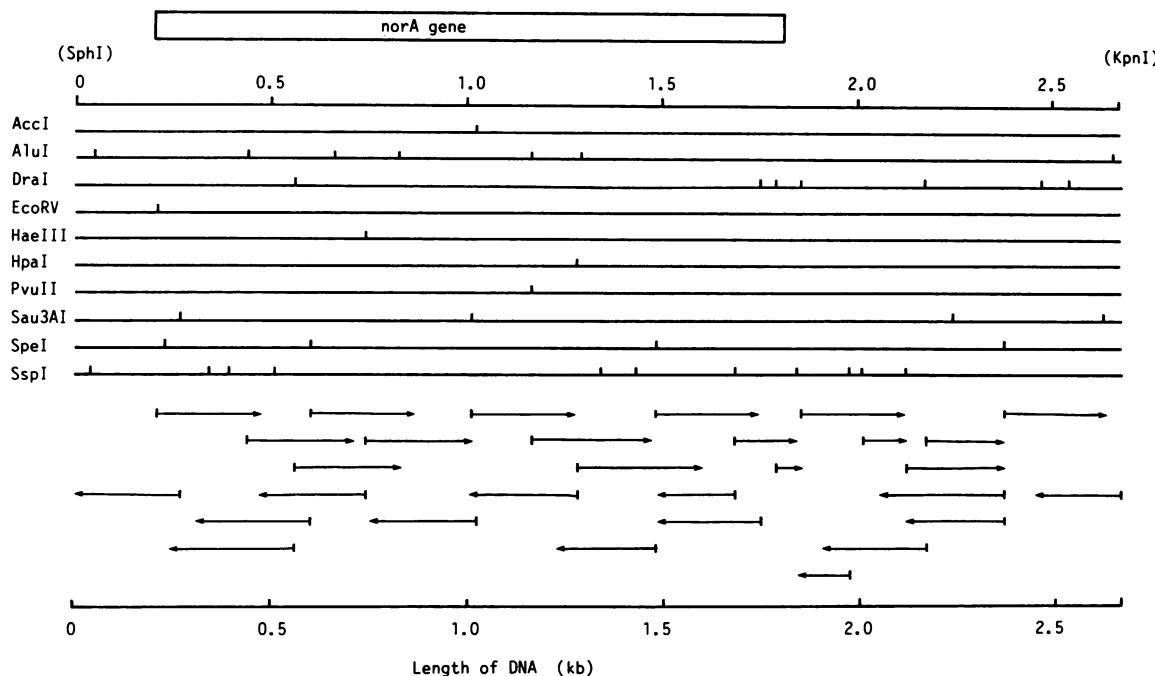


FIG. 2. Restriction map of the 2.7-kb *SphI*-*KpnI* fragment derived from pTUS829 and the strategy for determining the nucleotide sequence. Cleavage sites for restriction enzymes are shown as vertical lines. The arrows below the map indicate the direction and extent of sequence analysis.

10 20 30 40 50 60 70 80
 GCATGCCAAATGTGCAAATGACCATTGTCTAGACGAGAAAATTACCTATAAGCTCGTCAATTCCAGTGGCTCAGTAAT
 90 100 110 120 130 140 150 160
 ATGTTTTCTCGTATTGTTCTGTTAAATTGAAAATTAACTACACAGTCATGCACCATGCCGCTGACAG
 170 180 190 200 210 220 230 240
 ATGTAATGTTAAGTCTGGTCTGCAAAGGGTATACATTCAACGATATCTCTCTTCCAAACACTAGTAGT
 250 260 270 280 290 300 310 320
 ATAGTATGATTAATTGCAATTCTATGATCAATCCCCTTATTTAATATGTCATTAATTACAAATTAACTTAAATGGA
 330 340 350 360 370 380 390 400
 AAATAGTGTAAATTACAAGAAAAAAATTGTCAAATGTGCAATGTTGAATACAAATTAGAAACTTTACQAATATT
 -35 -10
 410 420 430 440 450 460 470 480
 TAGCATGAAATTGCAATCTGCTGGAAAAGAAGAATAAACAGCTTAAAGCATGACATGGAGAAAAAGGGTGGCATATG
 SD Net
 490 500 510 520 530 540 550 560
 AATAAACAGATTTGTCCTATATTAAATTCTGATTTTTAGGTATCGGTTAGTAATACCACTCTGGCTGT
 AsnLysGinIlePheValLeuTyrPheAsnIlePheLeuIlePheLeuGlyIleGlyLeuValIleProValLeuProVal
 570 580 590 600 610 620 630 640
 TTATTTAAAGATTTGGATTAACTGGTAGTTAGGATTACTAGTGTGCTGTTTGCCTTATCCTAAATGATTAT
 TyrLeuLysAspLeuGlyIleLeuThrGlySerAspLeuGlyLeuLeuValAlaAlaPheAlaLeuSerGlnMetIleIleSer
 650 660 670 680 690 700 710 720
 CGCCGTTTGTTGGTAGCTAGCTGACAAATTAGGGAAAGAAAATTATATGTTAGGATTAATTGGTTTCACTGGTCA
 ProPheGlyIleGlyThrLeuAlaAspLysLeuGlyLysLeuIleCysIleGlyIleLeuPheSerValSer
 730 740 750 760 770 780 790 800
 GAATTTATGTTGCACTGGCACAAATTTCGATTGATGTTAGGATTACTAGTGTGCTGTTAGGTTGATGGTATGGT
 GluPheMetPheAlaValGlyHisAsnPheSerValLeuMetLeuSerArgValIleGlyGlyNetSerAlaGlyNetVal
 810 820 830 840 850 860 870 880
 AATGCCCTGGTGTGACAGGTTAAATGCGACATTTCACCAAGGCCATCAAAAGCAAAACCTTGGCTATCGTACCG
 MetProGlyValThrGlyLeuAlaAspIleSerProSerHisGinLysAlaLysAsnPheGlyTyrMetSerAlaIle
 890 900 910 920 930 940 950 960
 TTATCAATTCTGGATTCACTTCTAGCATTATAATGTCATTGATTGATCAGATCGAAAGTCTACATCGTACCG
 IleAsnSerGlyPhelleLeuGlyProGlyIleGlyGlyPheMetAlaGluValSerHisArgMetProPheThrPhe
 970 980 990 1000 1010 1020 1030 1040
 GCAGGAGCATAGGATTCTAGCATTATAATGTCATTGATTGATCAGATCGAAAGTCTACATCGTACCG
 AlaGlyAlaLeuGlyIleLeuAlaPhelleSerIleValLeuIleHisAspProLysLysSerThrThrSerGlyPhe
 1050 1060 1070 1080 1090 1100 1110 1120
 CCAAAAGTTAGAGGCCAAATTGCTAACGGAAAATTAACTGGAAAGTCTTATTACACAGCTTATTTAACACTGTTATT
 GinLysLeuGluProGinLeuLeuThrLysIleAsnTrpLysValPhelleThrProValIleLeuThrLeuValLeuSer
 1130 1140 1150 1160 1170 1180 1190 1200
 CGTTTGGTTATCTGCATTGGAAACATTGTATTCACTACATACACAGCTGCAAGGTAATTATTACACCTGAAAGTAA
 PhelleLeuGlyIleLeuAlaPhelleSerIleLeuThrAlaAspLysValAsnTyrSerProLysAspIleSer
 1210 1220 1230 1240 1250 1260 1270 1280
 ATTGGCTATTACGGGTGGCGCTATTGGGGCACTTTCCAAATCTATTCTCGTAAATTATGAGTATTCTCGA
 IleAlaIleThrGlyIleGlyIlePheGinAlaLeuPheGinIleThrPhePheAspLysPheMetLysThrPheSerGlu

TABLE 1. Quinolone susceptibility of *S. aureus* and *E. coli* strains harboring the *norA* gene

Strain and plasmid	Functional <i>norA</i> gene	MIC (µg/ml) of drug ^a						
		Hydrophilic quinolones				Hydrophobic quinolones		
		ENX	NFLX	OFLX	CPFX	NA	OA	SPFX
<i>S. aureus</i>								
SA113	—	0.39	0.78	0.39	0.2	12.5	0.78	0.05
SA113(pTUS20)	+	25	50	6.25	12.5	25	1.56	0.2
<i>E. coli</i>								
HB101	—	0.05	0.025	0.0125	0.0063	1.56	0.39	0.0016
HB101(pTUS1)	+	0.78	0.78	0.1	0.1	3.13	0.39	0.0016
HB101(pTUS180)	+	1.56	1.56	0.1	0.1	3.13	0.39	0.0016
HB101(pTUS829)	+	1.56	1.56	0.1	0.1	3.13	0.39	0.0016
HB101(pTUS206)	+	1.56	1.56	0.1	0.1	3.13	0.39	0.0016
HB101(pTUS207)	—	0.05	0.025	0.0125	0.0063	1.56	0.39	0.0016
Partition coefficient ^b		0.06	0.07	0.11	0.06	3.39	0.85	0.91

^a ENX, Enoxacin; NFLX, norfloxacin; OFLX, ofloxacin; CPFX, ciprofloxacin; NA, nalidixic acid; OA, oxolinic acid; SPFX, sparfloxacin.

^b Partition coefficient in *n*-octanol-0.1 M phosphate buffer, pH 7.2.

1290 1300 1310 1320 1330 1340 1350 1360
 GTTAAACATTATAGCTTGGTCATTATTATTCAGTTGCTTAATATTATTAGTTTGCTAATGGCTATTGGTCAA
 LeuThrPhelleAlaTrpSerLeuLeuTyrSerValValValLeuIleLeuValPheAlaAsnGlyTyrTrpSerile
 1370 1380 1390 1400 1410 1420 1430 1440
 TAATGTTAACAGTTGTGCTTCATAGTTGATATGACGCCATTACAATTATTTCTAATATTGCT
 MetLeuIleSerPheValValPhelleGlyPheAspMetIleArgProAlaIleThrAsnTyrPheSerAsnIleAla
 1450 1460 1470 1480 1490 1500 1510 1520
 GGAGAAAGGCAAGCCTTGCAGCGGATTGAACATCGACATTACTAGATGGTAATTTCATAGTCCTTAATCGCAGG
 GlyGluArgIleGlyPheAlaGlyGlyLeuAsnSerThrPheThrSerMetGlyAsnPhelleGlyProLeuIleAlaGly
 1530 1540 1550 1560 1570 1580 1590 1600
 TGCGTTATTTGATGTACACATTGAAGCCAAATTATGGCTATAGGTGTTTCAAGCAGGTGTTGTTATTGTTTAA
 AlaLeuPheAspValHisIleGluAlaProIleTyrSerValSerAlaGlyValValIleValLeuIle
 1610 1620 1630 1640 1650 1660 1670 1680
 TTGAAAAGCAACATAGAGCAGAAATTGAAAGAACAAATATGTAGCATAAGTATTGGTGTATATTGATAAAAGTAAAG
 GluLysGinHisArgAlaLysLeuGluGinAsnNet
 1690 1700 1710 1720 1730 1740 1750 1760
 CGTAATATTATGAATGATTAGCATCGTTTCTATGAATTAAAGAAAAATTGATGCTTACATTAAAAGATTTC
 transcription terminator
 1770 1780 1790 1800 1810 1820 1830 1840
 GATTGACTAAATGTTTACTCTTTATTTAAATGTTATATGTAACAAAAAAATGATTTGAGTAATAAACATGTTACAAA
 1850 1860 1870 1880 1890 1900 1910 1920
 TATTACATTCTTTAAATGCAATCCACATACCTAACCTAACGTTAATGTTAAAGATGATAAAAATGAGTAAGG
 1930 1940 1950 1960 1970 1980 1990 2000
 AAATGTTGGTAAGGGATGACAGTAAAAAAATTATTTAGGTTGGCTGTAATATAACCGTTGGTTAAATTGTT
 2010 2020 2030 2040 2050 2060 2070 2080
 TATTAATATTAGCAACATGAAGATGCGCTGCTAACGTTAACGATCAAACAAATTACCGTTAACCGATAATGTC
 2090 2100 2110 2120 2130 2140 2150 2160
 CTGAAGACTTATAAAAGAAAATGGATTCTCAATATTCTAACACTGCTAACGATCTGAAGTGTGAAATGTTG
 2170 2180 2190 2200 2210 2220 2230 2240
 AGCAGAAATCATTAAACATCGTGTGAATGCAAAATAATCAAATTCTTAAACGACAAGAGTGCCTAAGTATTGCT
 2250 2260 2270 2280 2290 2300 2310 2320
 CGTTATGCAGATAAAGCATATCAATGATAATTATGGTTAGAAAAGATTCTAACGACAATCATGGTTAGAAA
 2330 2340 2350 2360 2370 2380 2390 2400
 TAAGACAATCATGGATAATTATGTTATTCAATGATAATTCAACTAGTAAGCAACATGTAAGTATTCAATCAAG
 2410 2420 2430 2440 2450 2460 2470 2480
 GCATAATAACGAAATAATAGATGGAACAGTGTATTCTAACGGATATACTGTTTATTGCAATAATTAA
 2490 2500 2510 2520 2530 2540 2550 2560
 AGGTGAAATCACTTATAAAATGATGAAATGTTATGTCAAATCACCACCGTACATGTTAAATGTTAATAG
 2570 2580 2590 2600 2610 2620 2630 2640
 TTCTGAAGAAGTATAATGAGGTGTTGAAATGGCTAAAAGAAAAGCACCGGATGCGTATGCTTGTGCAAAA
 2650 2660
 ATTAATACGAAGTTCATAGCTTGAG

(pTUS20) cells was determined by quantitating the cell-associated drug. Bacterial cells suspended at a density of 6×10^8 to 7×10^8 cells per ml were incubated with ^{14}C -enoxacin in the presence or absence of CCCP (0.1 mM) or ^{14}C -sparfloxacin at a concentration of 10 $\mu\text{g}/\text{ml}$ at 37°C, and 1-ml aliquots were centrifuged at the indicated times. Radioactivity associated with unwashed cells was measured by liquid scintillation counting.

Nucleotide sequence accession number. Nucleotide sequence accession number D90119 has been assigned to the sequences reported herein in the DDBJ, EMBL, and GenBank data bases.

RESULTS

Quinolone susceptibility of *S. aureus* and *E. coli* strains harboring the norA gene. *S. aureus* SA113(pTUS20) harbor-

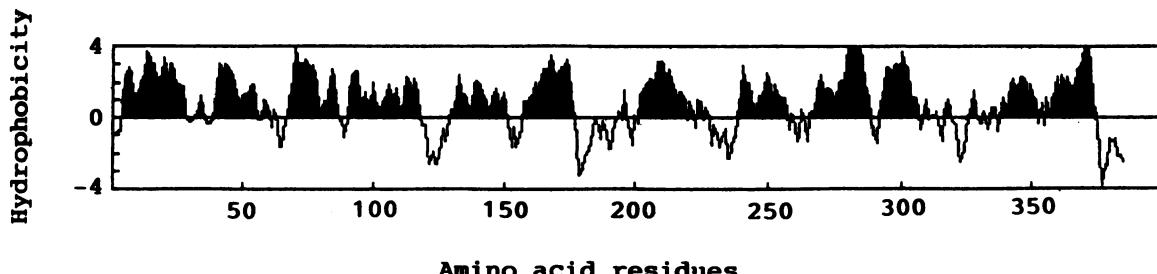


FIG. 4. Hydropathic profile of the predicted amino acid sequence of the NorA polypeptide according to the algorithm of Kyte and Doolittle (19).

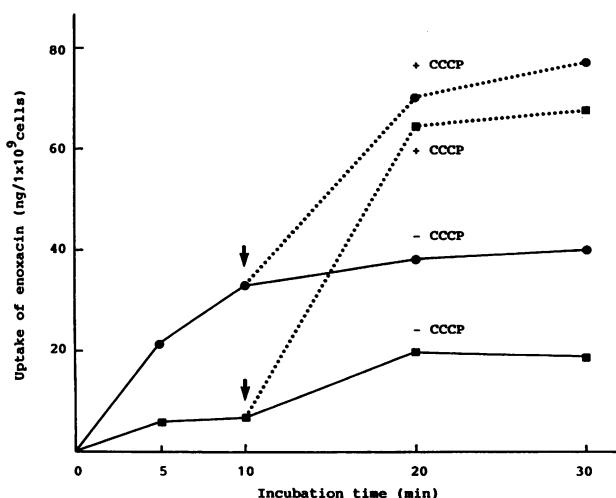


FIG. 5. Uptake of ¹⁴C-enoxacin by *S. aureus* cells with (+) or without (-) CCCP. ●, *S. aureus* SA113, the parent strain; ■, *S. aureus* SA113(pTUS20) containing the *norA* gene. CCCP was added to a final concentration of 0.1 mM at the time indicated by the arrows.

ing a plasmid carrying the staphylococcal *norA* gene was 16 to 64 times more resistant to relatively hydrophilic quinolones such as enoxacin, norfloxacin, ofloxacin, and ciprofloxacin than the parent quinolone-susceptible strain, SA113, whereas the former strain was only 2 to 4 times more resistant to relatively hydrophobic quinolones such as nalidixic acid, oxolinic acid, and sparfloxacin (Table 1). *E. coli* strains containing one of the plasmids carrying the *norA* gene (pTUS1, pTUS180, pTUS829, and pTUS206) were 8 to 64 times more resistant to the hydrophilic quinolones than the parent quinolone-susceptible strain, HB101, but as susceptible to or only 2 times more resistant to hydrophobic quinolones. The *E. coli* strain harboring the plasmid carrying the nonfunctional deleted *norA* gene, pTUS207, was not resistant to quinolones at all. The *S. aureus* and *E. coli* strains resistant to quinolones because of the *norA* gene remained susceptible to antibiotics such as tetracycline, gentamicin, and erythromycin (data not shown).

Nucleotide sequence and identification of the *norA* gene. The nucleotide sequence of the 2.7-kb *Sph*I-*Kpn*I fragment containing the *norA* gene from pTUS29 was determined by the strategy shown in Fig. 2. The nucleotide sequence contained only one open reading frame (nucleotides 478 to 1641) long enough to encode a polypeptide of 388 amino acids (Fig. 3). This open reading frame was considered to encode the NorA polypeptide, because a protein with an estimated molecular weight of about 49,000 was detected upon DNA-directed translation with *Bam*HI-cleaved pTUS829 DNA containing the entire *norA* gene, but was not observed on that using *Bam*HI-cleaved pTUS207 DNA without the functional *norA* gene (data not shown). The molecular weight of 42,264 calculated from the deduced amino acids of the *norA* gene agreed with the experimental value.

Putative promoter sequences were found at nucleotides 373 to 378 (TACAAT) and nucleotides 349 to 354 (TTGTCA), which well match the consensus sequences (TATAAT and TTGACA) for the -10 and -35 regions of *E. coli* promoters. The sequence for ribosomal binding (AAAA GAGGT) is located 7 to 15 bp upstream of the initiation codon, ATG, which closely matches the Shine-Dalgarno

TABLE 2. Amino acid composition of the deduced NorA polypeptide

Amino acid	No. of residues (% total) ^a
Ala	28 (7.2)
Val	28 (7.2)
Leu	47 (12.1)
Ile	45 (11.6)
Pro	14 (3.6)
Met	17 (4.4)
Phe	36 (9.3)
Trp	3 (0.8)
Gly	40 (10.3)
Ser	30 (7.7)
Thr	15 (3.9)
Cys	1 (0.3)
Tyr	13 (3.4)
Asn	13 (3.4)
Gln	9 (2.3)
Asp	10 (2.6)
Glu	9 (2.3)
Lys	19 (4.9)
Arg	5 (1.3)
His	6 (1.6)

^a Total number of amino acid residues is 388. The molecular weight of NorA is 42,264.9.

sequence (TAAGGAGGT). When the 387-bp *Alu*I fragment (nucleotides 55 to 441) containing the putative promoter was ligated with the *Sma*I site of pKK232-8 containing a promoterless chloramphenicol acetyltransferase gene and then introduced into *E. coli* HB101, the resultant transformant exhibited resistance to chloramphenicol, indicating that this DNA fragment has promoter activity (data not shown). An inverted repeat, which might act as a transcription terminator, was found at nucleotides 1700 to 1742.

Properties of the NorA polypeptide. The amino acid composition of the deduced NorA polypeptide is shown in Table 2. This polypeptide is rich in hydrophobic amino acids such as Val, Leu, Ile, Phe, and Met; 173 (45%) of the 388 amino acids are hydrophobic. The hydropathic profile calculated by using the algorithm of Kyte and Doolittle (19) is shown in

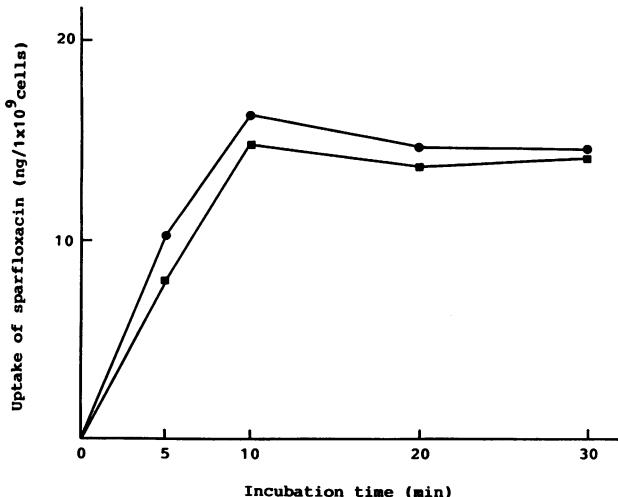


FIG. 6. Uptake of ¹⁴C-sparfloxacin by *S. aureus* cells. ●, *S. aureus* SA113, the parent strain; ■, *S. aureus* SA113(pTUS20) containing the *norA* gene.

NorA	1	<u>MNKQIFVLYFNIFLIFLGIGLVLIPVLPVKDL-GLTG-SD-LGLLVAAF-ALSOQIISPFGGT</u>	10	20	30	40	50	60
Tn10	1	<u>MNSSTKIALVITLLDAMGIGLIMPVLPVTLREFIASEDIANHFGVLLALY-ALMQVIFAPWLCK</u>	**	*	****	*	*	*
Xyl	275	<u>VGVIVIGVMSLIPQQFVGIVNVLYYAAPEVFKTL-G-AST-D-IALLQTIIIVGVINLTFTVLA1M</u>	**	*	**	*	**	*
Glc	65	<u>WSLSVAIF-SVGGMI-GSFSVG</u>						
NorA	61	<u>L-ADKLGKK-L-II-CIGL-I-LFSVSEFM-FA-VGHNFSVLMLSRVIGGMSAGMVMPGVT-GL</u>	70	80	90	100	110	
Tn10	64	<u>M-SDRFGR-P-VL-LLSL-I-GASLDYLL-LA-FSSALWMLYLGRLLSGIT-GATGAVAA-SV</u>	**	*	*	*	*	*
Xyl	335	<u>T-VDKFGRKPLQIIGALGMAIGMPSLGTAF-YT-QAPGI-VALSMLFYVAAFAMSWGPVCWVL</u>	**	*	*	*	*	*
Glc	85	<u>LFVNRFGR-NSML-MMNL-L-AFVSAVLMGFSKLGKSFEMLILGRFIIGVYCGLTTGFVP-MY</u>						
NorA	116	<u>IADISPShQAKNFGY-MSAIINSGFILGP--GIGGFMAEVSHRMPPFYFAGALGILAF-IMSIIV</u>	120	130	140	150	160	170
Tn10	118	<u>IADTTSASQRVKWFGW-LGASFGLIAGP--IIGGFAGEISPHSPFFFIAALLNIVTF-LVVMF</u>	**	*	*	***	*	*
Xyl	395	<u>LSEIIFPNAIRGKALAIAVAAQWLANYFVSW--TFP-MM-DKNSWLVAFHNGFSYWIYGMGV</u>	**	*	*	*	*	*
Glc	144	<u>YGEVSPTAFRG-ALGTLHQLGIVVGLIAQVPGLDSTMGNKDLW-PLLLSIIF-IPAL-LQCIV</u>						
NorA	176	<u>LIHDPKKSTTSQPKLEPQLLTKINW-KVFit-PViLTlVLSFGL-SAF-ETLYSLYTADKVNY</u>	180	190	200	210	220	230
Tn10	178	<u>WFRETKNTRDNNTDTEVGVETQSNSVYITLFKTMPIILLIYFSAQI</u> <u>QI</u> <u>PATVWVLFTENRFGW</u>	*	*	*	*	*	*
Xyl	455	<u>AALPMW</u>	*					
Glc	204	<u>LPF</u>						
NorA	236	<u>SPKDIsIAITGGGIFGALFOIYFFDKFMKYFSELTIFIAWSLLSVVVLLVFANGYWSIMLIS</u>	240	250	260	270	280	290
Tn10	242	<u>NSMMVGFSLAGLGLLHSVQAFVAGRIATKWGEKTAVLLGFIADSSAFAFLAFISEGW-LVFPV</u>	*	*	**	*	*	*
NorA	300	<u>FVVFIGFDMIRPAITNYFS-NIAGERQGFAGGLNSTFTSMGNFIGPLIAGALFD--VHI-EAPI</u>	320	330	340	350		
Tn10	306	<u>LILLAGGGIALPALQGVMSIQTKSHQQGALQGLLVLSLTNATGVIGPLLFAVLYNHSLPIWDGW</u>	*	**	*	***	*	*
NorA	360	<u>YMAIGVSLAGVIVLIEKQHRAKLKEQNM</u>	370	380				
Tn10	371	<u>WI-IGLAFYCIIILLSMTFMLTPQAQGSKQETSA</u>	**	**				

FIG. 7. Comparison of the amino acid sequence of the NorA polypeptide, TetA protein (28), xylose transport protein (21), and glucose transport protein (1). Only a homologous part of the sequences detected by the program SEQFP (34) in the IDEAS system (17) is aligned. Identical amino acids with the NorA polypeptide have asterisks above the sequences. Possible hydrophobic membrane-spanning regions are underlined.

Fig. 4. There are 12 hydrophobic regions, most of which are long enough to span the membrane.

Uptake of quinolones by *S. aureus* cells. The uptake of quinolones was examined in *S. aureus* SA113 and SA113(pTUS20). The uptake of a hydrophilic quinolone, enoxacin, by *S. aureus* SA113(pTUS20) cells was about 50% that by *S. aureus* SA113 cells, and the former increased to almost the same level as the latter when CCCP was added (Fig. 5). In contrast, the uptake of a hydrophobic quinolone, sparfloxacin, was similar in the two strains (Fig. 6).

DISCUSSION

Nucleotide sequence analysis of the staphylococcal *norA* gene revealed that it can encode 388 amino acids with a molecular weight of 42,265 and is preceded by a putative promoter region. The promoter sequence of the *norA* gene resembles the *E. coli* consensus promoter sequence and is functional in *E. coli* cells when checked by the promoterless chloramphenicol acetyltransferase gene. No similar se-

quence was found in the EMBL and GenBank data bases. Therefore, the *norA* gene is different from the *gyrA* or *gyrB* gene, contrary to what was expected from the early Southern blot analysis (33). Reinvestigation of the previous experimental results disclosed that a possible contaminant in the *norA* gene fragment used as a probe had hybridized with the bands of vector DNA electrophoresed close to the bands of the *gyrA* and *gyrB* gene fragments and caused us to misinterpret the results.

The NorA polypeptide is rich in hydrophobic amino acids; 45% of the total amino acids are hydrophobic. There are some proteins showing low identity in amino acids with the NorA polypeptide: tetracycline resistance protein (TetA) in *E. coli*, 24% (28); xylose transport protein (*E. coli*), 21% (21); and glucose transport protein (rat), 24% (1). The NorA and the homologous proteins have 12 hydrophobic regions which can span the cell membrane. Proteins responsible for transport are generally believed to traverse the cell membrane several times forming a specific transport channel (3). The

NorA polypeptide resembles the TetA protein to a greater extent than the sugar transport proteins in similarities of the hydrophobic regions (Fig. 7). The NorA and TetA proteins have nine and eight hydrophobic regions, respectively, in which they contain one or two proline residues. It has been reported that proline residues are found in membrane-spanning regions of nearly all transport proteins and provide the reversible conformational change requisite for the opening and closing of a transport channel (3). In addition, the eight membrane-buried proline residues are conserved between the two proteins. These findings suggest that the NorA protein is a transport protein with an evolutionary relationship to the TetA protein, which has proved to be an energy-dependent efflux pump of tetracycline (6, 24, 28).

In fact, the uptake of hydrophilic quinolone, enoxacin, was lower in *S. aureus* SA113(pTUS20) cells harboring a plasmid carrying the *norA* gene than that in *S. aureus* SA113 lacking the plasmid (Fig. 5). Since the enoxacin uptake of *S. aureus* SA113(pTUS20) increased to almost the same level as that of *S. aureus* SA113 by the addition of CCCP, the NorA polypeptide seems to be an energy-dependent efflux pump of quinolones. Interestingly, the uptake of a hydrophobic quinolone, sparfloxacin, was not affected at all by the *norA* gene (Fig. 6). This suggests that only hydrophilic quinolones, but not hydrophobic ones, may excrete through the pump. This interpretation is consistent with the fact that *S. aureus* and *E. coli* strains containing a plasmid carrying the *norA* gene are resistant to hydrophilic quinolones but are not resistant or are less resistant to hydrophobic ones.

Cohen et al. have shown that there is an energy-dependent efflux pump of norfloxacin in the inner membrane of quinolone-susceptible *E. coli* strains, in which hydrophilic quinolones but not hydrophobic ones inhibit the transport of norfloxacin (5). Quinolone-susceptible *S. aureus* SA113 also has an energy-dependent efflux pump of enoxacin (Fig. 5). Therefore, an important question arises as to whether the NorA protein is identical with the protein constituting the efflux pump of quinolone-susceptible *S. aureus* and whether quinolone resistance is due to the overproduction of the latter protein. Cloning and sequencing of the allele of the *norA* gene in quinolone-susceptible *S. aureus* strains will answer this.

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